

ELASTOTIC MATERIAL AND ELASTIC FIBERS IN AGED SKIN: AN ULTRASTRUCTURAL STUDY WITH CONVENTIONAL AND TANNIC ACID STAIN

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Abstract. Elastotic material and aged elastic fibers were studied with an electron microscope, using both conventional and tannic acid stain. (1) A relatively large part of the fibrous form, and a lesser part of the amorphous form stained positive with the tannic acid stain. (2) Aged elastic fibers showed some age-related findings with both conventional and tannic acid staining. It is suggested that the elastotic material originates from the elastic fibers and progresses from the fibrous to the amorphous form.

Key words: Aged elastic fibers; Elastotic material; Tannic acid stain

Many studies have been published on the ultrastructure of solar elastosis, including the description of its origin and process of degeneration (1-8).

As to its origin, the traditional view is that the elastotic material of solar elastosis forms as a result of degeneration, either of collagen (2-4), or of elastic tissue (1, 7), or of both (8). In contrast to the traditional view, several authors have suggested that it could be synthesized by sun-damaged fibroblasts rather than forming as a purely degenerative process of the connective tissue (5, 6).

Most of these studies have carried out using the conventional electron stain (that is, uranyl acetate and lead citrate). Some investigators have used phosphotungstic acid (PTA) (2, 5) which is known as one of the staining methods for elastic fibers. The PTA, however, stained not only elastic fibers, but also collagen fibers (9) and could not be used effectively with uranyl acetate and lead citrate as counterstains (10).

On the other hand, a staining method using a tannic acid as fixative for elastic fibers, developed by Mizuhira et al. (11), and improved for Epon-embedded thin sections by Kajikawa et al. (12) proved specific solely for elastic fibers. In addition, the counterstaining can be used in this method (12). It

may be reasonable to apply this method for a study on solar elastosis. We have found that the elastotic material showed an affinity for the tannic acid, as did normal elastic fibers.

The purpose of this paper is to describe the ultrastructural findings in the elastotic material as well as those in elastic fibers, using both conventional and the tannic acid stain, and discuss the origin and process of its formation.

MATERIALS AND METHODS

Specimens were taken from the buttock and neck of 4 farmers (62 to 82 years old) affected by cutis rhomboidalis nuchae. The specimens were fixed at 4°C for 1 h in 2% glutaraldehyde buffered with 0.2 M phosphate (pH 7.4). After thorough rinsing they were immersed in 1% osmium tetroxide buffered with 0.2 M phosphate (pH 7.4) for 1 h and then dehydrated in graded ethyl alcohol concentrations and embedded in Epon 812. Ultrathin sections were cut with a Porter-Blum MT-2 ultramicrotome. Some sections were stained with a uranyl-acetate solution for 1 h and with Reynolds' lead citrate for 10 min. Others were stained with a tannic acid-uranyl acetate solution¹ (12) for 10 min and with Reynolds' lead citrate for 10 min. They were examined with a Hitachi HS 9 electron microscope.

RESULTS

(1) Aged skin from the buttock

(a) *Conventional stain (uranyl acetate and lead citrate).* The elastic fibers consisted of an amorphous matrix and microfibrils. The former was located centrally and microfibrils were distributed around the periphery. There were relatively large elec-

¹ The tannic acid-uranyl acetate solution was prepared as follows: 0.2 g of tannic acid and 0.5 g of paranitrophenol were dissolved completely in 35 ml of distilled water with the aid of heat. After cooling, 0.2 to 0.4 ml of 5% uranyl acetate was added to this solution.

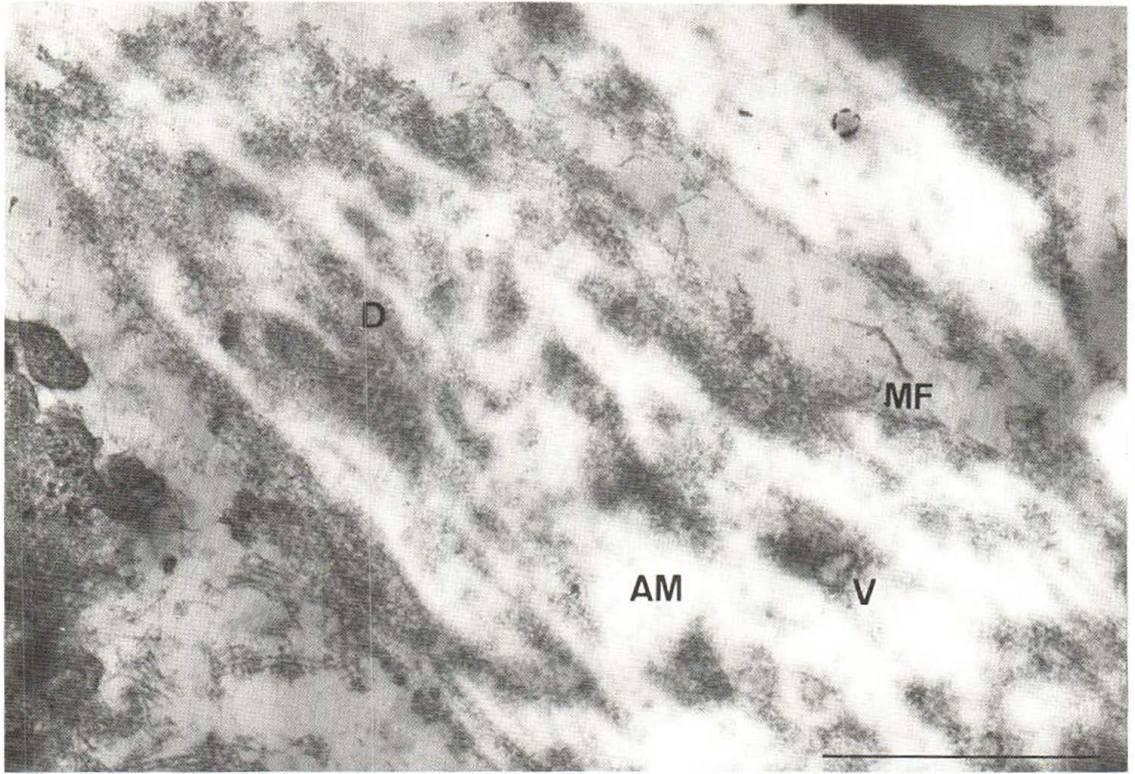


Fig. 1. Aged elastic fiber (conventional stain). Electron-lucent amorphous matrix (AM) and relatively large electron-dense areas (D), some of which contain various sized

vesicular organelles (V) can be seen. A few microfibrils (MF) are seen at the periphery of the fiber. Scale = 1 μ m.

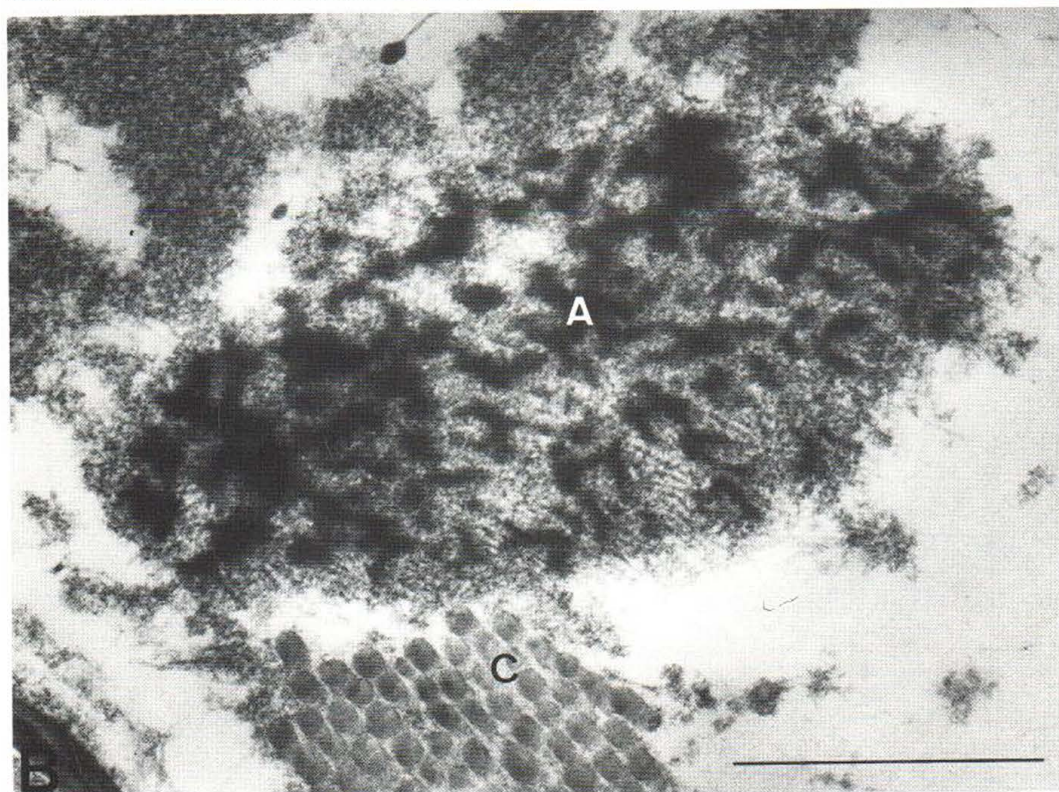
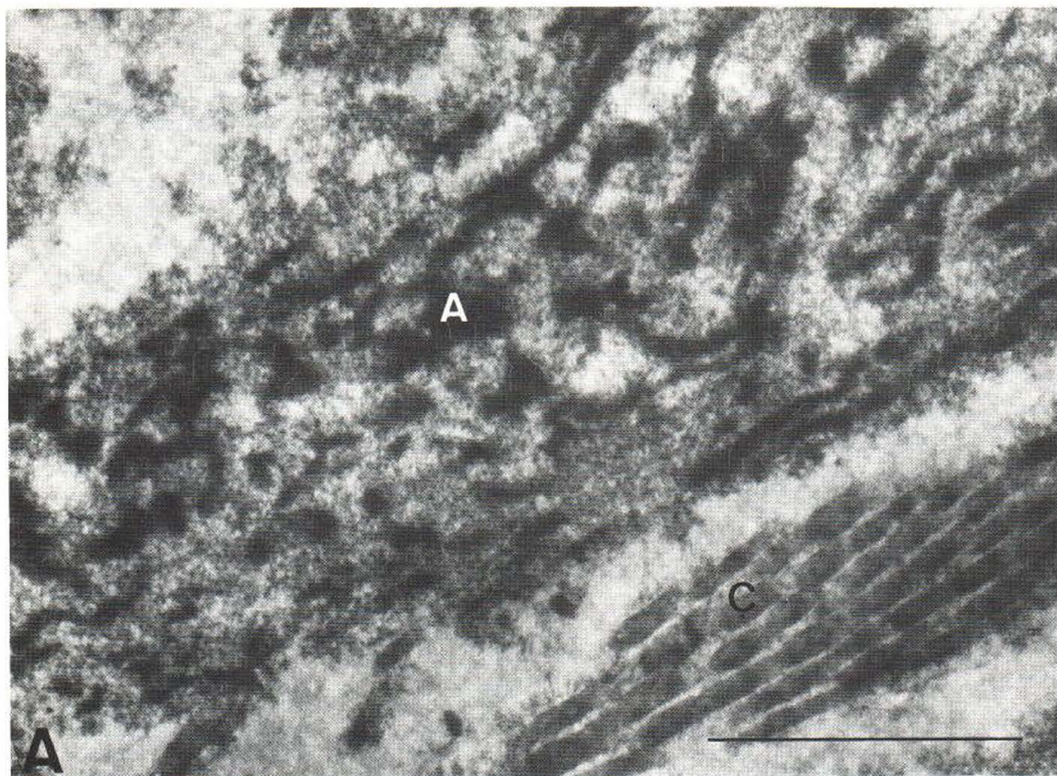
tron-dense areas in the amorphous matrix with orientation parallel to the long axis of the fiber; some of these contained various sized vesicles which were regarded as an age-related change (Fig. 1) (13, 14).

(b) *Tannic acid stain (tannic acid–uranyl and lead citrate)*. The amorphous matrix showed marked affinity for the tannic acid and stained black, while microfibrils as well as the electron-dense areas seen in the conventionally stained section showed no affinity and stained slightly dark because of the counterstains with uranyl acetate and lead citrate. In longitudinal sections, the amorphous matrix was seen as a long and broad dense material, which ran parallel to the long axis of the elastic fiber (Fig. 2a). In transverse sections, it was intermingled with the microfibrils and slightly dark material in a reticular pattern (Fig. 2b). The other tissue and cell components such as collagen fibers, fibroblasts, and blood vessels showed no affinity for the tannic acid stain.

(2) *Altered skin from the neck*

(a) *Conventional stain*. Two forms of elastotic material could be distinguished—fibrous, and amorphous. The fibrous form resembled the mature elastic fiber in its ultrastructural pattern. It was characterized by numerous, various sized, irregular, electron-dense inclusions and an electron-lucent matrix (Fig. 3). Microfibrils were seen at the periphery of the fibers. The amorphous form was characterized by moderately electron-dense material consisting mainly of a mixture of fine granular and amorphous components (Fig. 4). Few microfibrils were seen at the periphery. Sometimes, there is a transitional

Fig. 2. Aged elastic fiber (tannic acid stain). (A) A longitudinal section. A long and broad dense material which is regarded as the amorphous matrix (white A) runs parallel to the long axis of the elastic fiber. (B) A transverse section. The dense material (white A) is intermingled with the microfibrils and slightly dark material in a reticular pattern. Scale = 1 μ m each. C: collagen fibers.



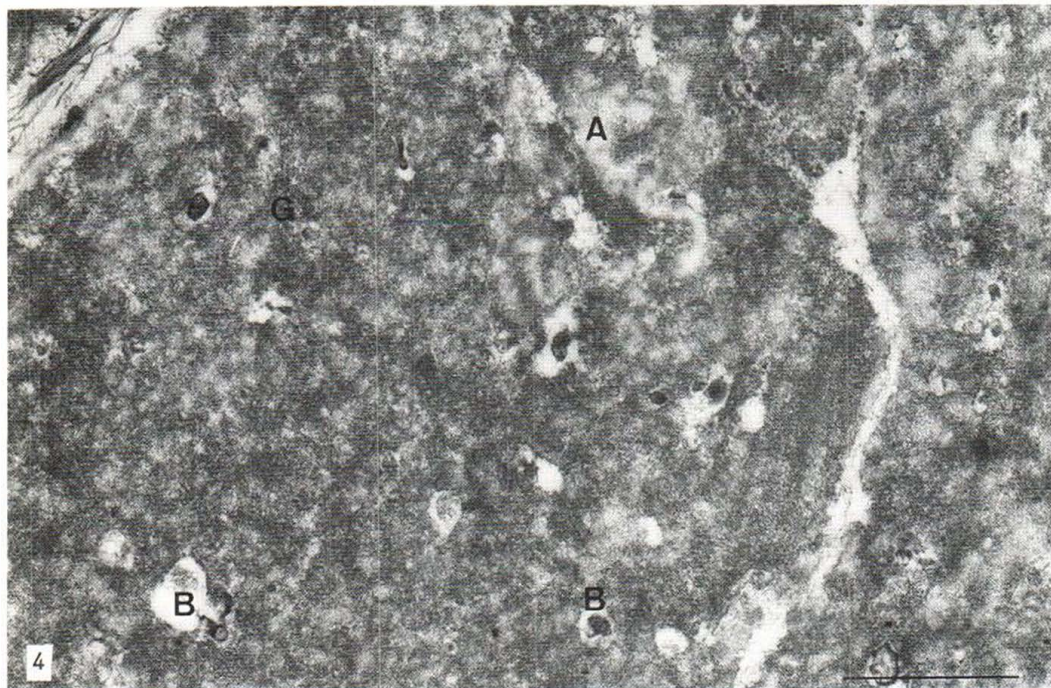
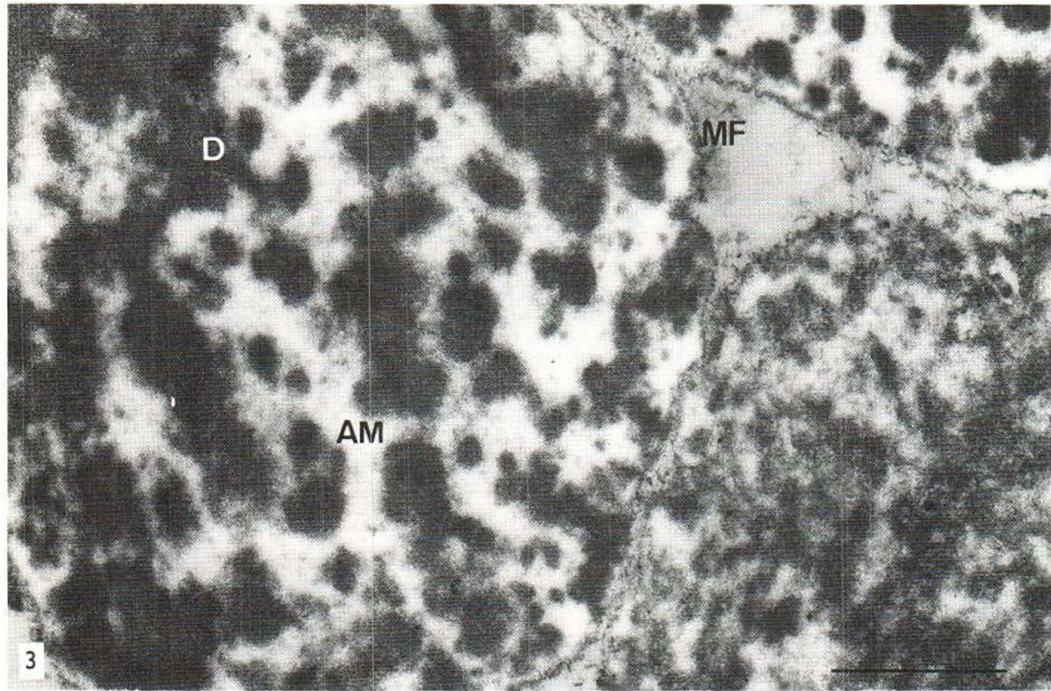


Fig. 3. A fibrous form of elastotic material (conventional stain). Numerous, various sized, and irregular electron-dense inclusions (white *D*) and an electron-lucent matrix (*AM*) can be seen. Microfibrils (*MF*) are present at the periphery. Scale = 1 μ m.

Fig. 4. An amorphous form of elastotic material (conventional stain).

A moderately electron-dense material consisting mainly of a mixture of fine granular (*G*) and amorphous (*A*) components can be seen. Round bodies (*B*) of varying size and electron density are scattered in this material. No microfibrils are present at the periphery. Scale = 1 μ m.

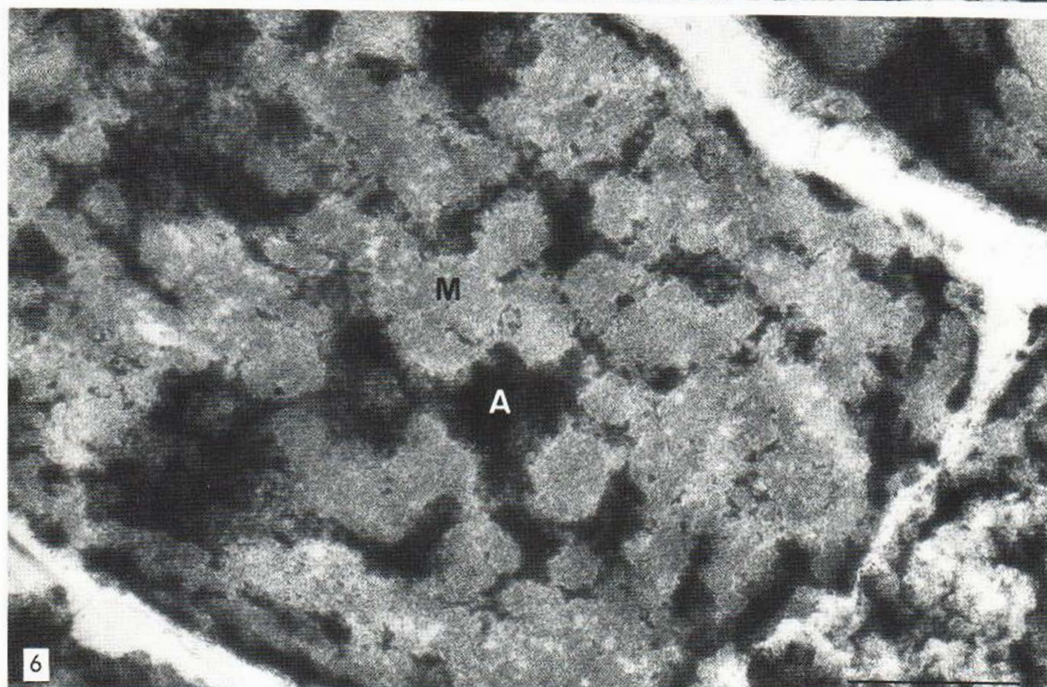
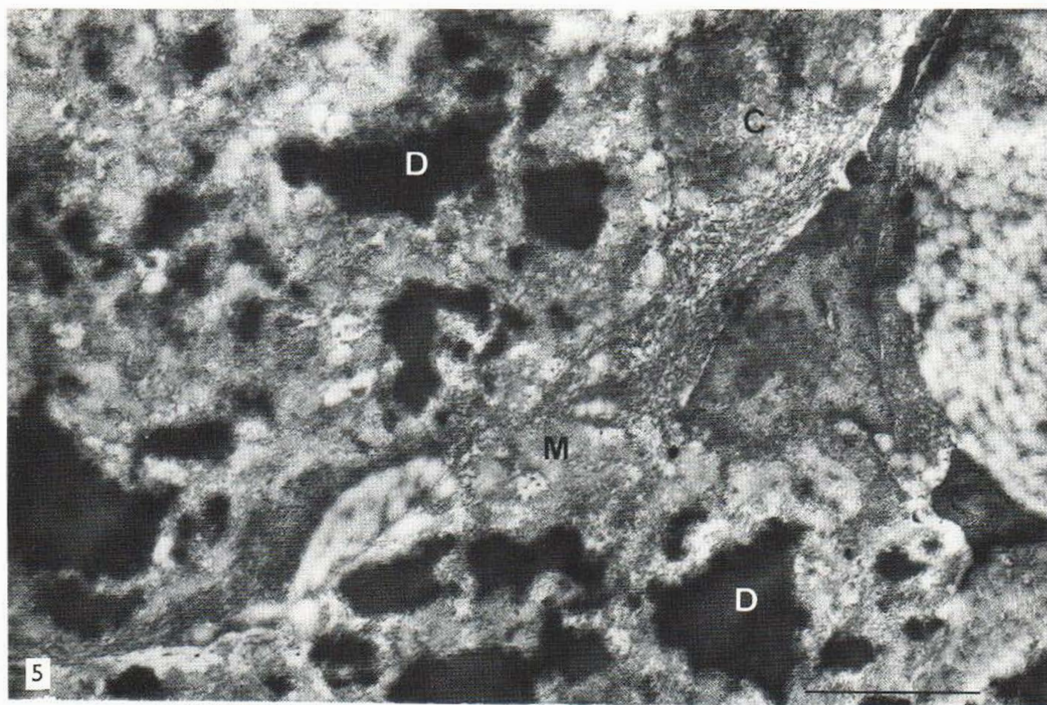


Fig. 5. A transitional form between the fibrous and the amorphous form (conventional stain). Irregular electron-dense inclusions (*white D*) and the moderately electron-dense matrix (*M*) can be seen. Scale = 1 μm . *C*: collagen fibers.

Fig. 6. A fibrous form of elastotic material (tannic acid stain). Electron-dense areas (*white A*) surround the fine granular material (*M*), forming several segments. Scale = 1 μm .

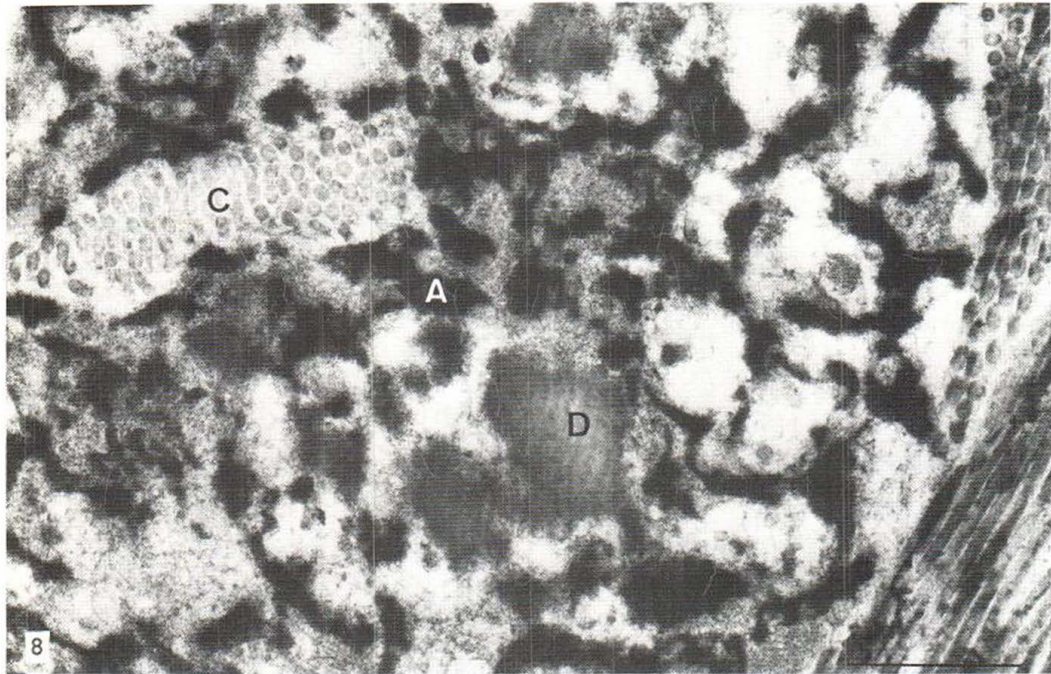
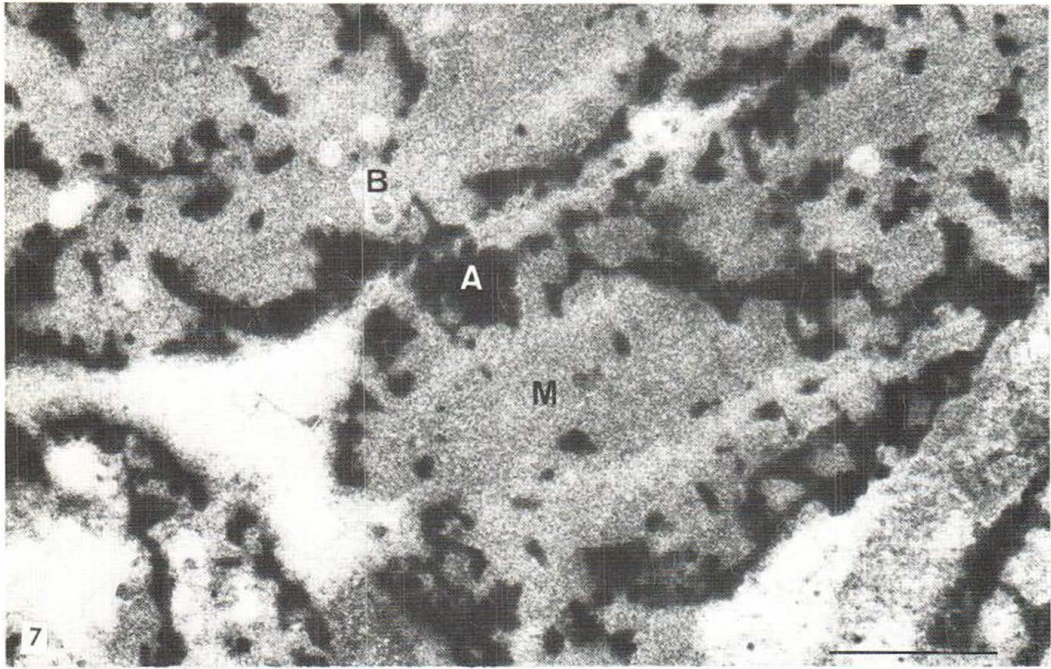


Fig. 7. An amorphous form of elastotic material (tannic acid stain). Electron-dense areas (white *A*) are sparse in the fine granular material (*M*). No segments of the fine granular material can be seen. Scale = 1 μm . *B*: round body.

Fig. 8. A transitional form between the fibrous and the amorphous form of elastotic material (tannic acid stain). A relatively large part of the electron-dense areas (white *A*) can be seen. Scale = 1 μm . *C*: collagen fibers. *D*: An area corresponding to the irregular electron-dense inclusions in sections stained with the conventional stain.

form between the fibrous and the amorphous form, showing irregular electron-dense inclusions and the moderately electron-dense matrix (Fig. 5).

(b) *Tannic acid stain.* In the fibrous form of the elastotic material, several electron-dense areas arranged in line, parallel to the long axis were seen among the matrix of the fine granular material, lightly stained with the counterstain. Transverse sections showed that the electron-dense areas surrounded the fine granular material, forming several segments (Fig. 6). In the amorphous form and the transitional form between the fibrous and the amorphous form, the electron-dense areas were more sparse and arranged more irregularly than in the fibrous form. These areas were surrounded by the matrix consisting of the fine granular material and the fine reticular filaments (Figs. 7 and 8). There were several spaces or interstitial areas among the matrix.

DISCUSSION

The ultrastructural findings of elastic fibers in aged skin reported here were similar to those described in some previous reports, using both transitional and tannic acid stain (13, 14). In comparison with those in young skin, the elastic fibers in aged skin stained with the transitional stain were found to contain fewer microfilaments, more electron-dense inclusions having the appearance of vesicles in the amorphous matrix, and the occurrence of fragmentation and disintegration of the fiber. With the tannic acid staining, the fibers in young skin stained homogeneously black, while those in aged skin stained reticular, possibly because of the decrease in the matrix (13, 15).

Some previous reports described how two forms of the elastotic material, fibrous and amorphous, could be distinguished (1, 8). These findings are similar to those demonstrated in the present studies. The finding of the elastotic material stained with the tannic acid has revealed new characteristics of the material. It has also been revealed that relatively large areas staining positive for tannic acid are present in the fibrous form, while there are fewer such areas in the amorphous form. Since the only amorphous matrix in the elastic fiber stains positive with this stain (6), it is assumed that the areas of the elastotic material staining positive for tannic acid have the same nature as the amorphous

matrix (clastin) of the elastic fiber. This may be additional proof that the elastotic material is derived from elastic fibers. In addition, the fact that the areas staining positive for tannic acid decreased in the order of the fibrous, the transitional, and the amorphous form of the elastotic fibers, suggests that the elastotic changes progress in this order.

We reject the hypothesis (5) that the elastotic changes start with the appearance of homogeneous and fine filamentous material among collagen fibers or near fibroblasts, since these materials are also seen in unexposed aged skin (Fig. 2*b*) and contain no material staining positive for tannic acid.

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